Nitrification and denitrification as sources of gaseous nitrogen emission from different forest soils in Changbai Mountain

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Abstract: The contributions of nitrification and denitrification to N_2O and N_2 emissions from four forest soils on northern slop of Changbai Mountain were measured with acetylene inhibition methods. In incubation experiments, 0.06% and 3% C_2H_2 were used to inhibit nitrification and denitrification in these soils, respectively. Both nitrification and denitification existed in these soils except tundra soil, where only denitrification was found. The annually averaged rates of nitrification and denitrification in mountain dark brown forest soil were much higher than that in other three soils. In mountain brown coniferous soil, contributions of different processes to gaseous nitrogen emissions were Denitrification N_2O > Nitrification N_2O > Denitrification N_2. The same sequence exists in mountain soddy soil as that in the mountain brown coniferous soil. The sequence in mountain tundra soil was Denitrification N_2O > Denitrification N_2.

Key words: Nitrous oxide; N_2; Nitrification; Denitrification; Forest soil; Acetylene inhibition method

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Introduction

Nitrous oxide (N_2O) is an important greenhouse gas. Its global warming potential is about 206 times that of CO_2 and its lifetime in atmosphere is 120 years. From our current knowledge, forest ecosystem is an important source of N_2O. The estimated N_2O emission from forest ecosystem was about half of that from terrestrial ecosystems in the world (IPCC 1992; Kreileman & Bouwman 1994).

Forestry area in China was 1.337×10^8 hm^2 and percentage of forest cover was 13.92%(Chinese Ministry of Forestry 1994). Forest ecosystem plays an important role in C and N cycling of terrestrial ecosystem. However, only few studies on emissions of greenhouse gases from Chinese forest ecosystem have been conducted up to now. In order to understand the contribution of Chinese forest ecosystem to the global budget of greenhouse gases, more researches are needed to study fluxes and biological processes of some important gases.

The nitrification and denitrification are known as major biological processes of N_2O production. However, the contributions of these two processes to N_2O production are difficult to measure in field conditions. In this study, gaseous nitrogen emissions through nitrification and denitrification in four types of fresh soils in forest ecosystems of Changbai Mountain, China, were measured with acetylene (C_2H_2) inhibition technique.

Materials and methods

Experimental sites

Changbai Mountain is located in Jilin Province, China (41°23′~42°36′N, 126°55′~129°E). From the summit to the foot, there is vertical zonality of several different forest ecosystems. Some characteristics of these forest ecosystems are listed in Table 1.

Incubation of soils

a) Soil sampling: The 8-10 repetitions of soils were sampled at 0-10 cm depth within a 15×15 m^2 area from four experimental sites A, B, C and D respectively. Soil samples were stored in plastic bags and transported to laboratory within 2 days.

b) Inhibition efficiencies of different C_2H_2 concentrations on N_2O production: 30 g sieved (2 mm) soil were incubated in 300 mL incubation bottles at 23 °C. The C_2H_2 concentrations in headspace of bottles were adjusted to 0, 0.01%, 0.03%, 0.06%, 0.12%,

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0.3%, 1% and 3% in each treatment respectively, four repetitions for each treatment. The N_2O concentration of air samples from the headspaces at 24 and 48 hours of incubation were measured with GC.

c) Measurement of N_2O production by nitrification and denitrification: 30 g sieved (2 mm) soil was incubated in a 300 mL culture bottle at 23 °C. Three treatments were conducted: None C_2H_2, 0.06%(v/v) C_2H_2, and 0.3%(v/v) C_2H_2 in headspace of bottle. N_2O concentrations of air samples from the headspaces at 48 h of incubation were measured with GC.

Analysis of N_2O concentrations

A gas chromatograph (Shimazu GC-14A) was operated with an electron capture detector (ECD) at 300 °C, a Porapak-Q precolumn (1 m in length) and a analytical column (2 m in length and 0.5 cm in diameter) at 60 °C, and a carrier gas (N_2) at the flow rate of 60 mL·min^{-1}. A 10-port valve permitted the heavier water vapor and other heavier components to be back-flushed from the precolumn at 0.98 min after injection of samples, whereas N_2O and lighter gases were separated on the analytical column.

Table 1. Some characteristics of four experimental sites in Changbai Mountain

	Site A	Site B	Site C	Site D
Forest type	Broad-leaved Korean pine mixed forest	Coniferous forest	Birch forest	Alpine tundra
Altitude (m)	740	1280	1850	2200
Soil type	Dark brown forest soil	Brown coniferous forest soil	Soddy forest soil	Tundra soil
Vegetation	*Pinus koraiensis* *Tilia amurrensis* *Fraxinus mandshurica*	*Picea jezoensis* *Abies nephrolepis* *P. koyamai var.koraiensis*	*Betula Ermanni* *Pinus pumila*	*Rhododendron aureum* *Phyllodoce caerulea*
Annual mean temperature (℃)	3.3	-0.7	-3.1	-7.3
Annual precipitation (mm)	800-700	1000-1100	1100-1000	1300-1100
Soil pH (KCl/H_2O)	4.75/5.67	4.40/5.55	3.91/4.97	4.33/5.54

Results

Optimal C_2H_2 concentration of nitrification inhibition

Fig. 1 showed the N_2O production of dark brown forest soil under different C_2H_2 concentration at 24,

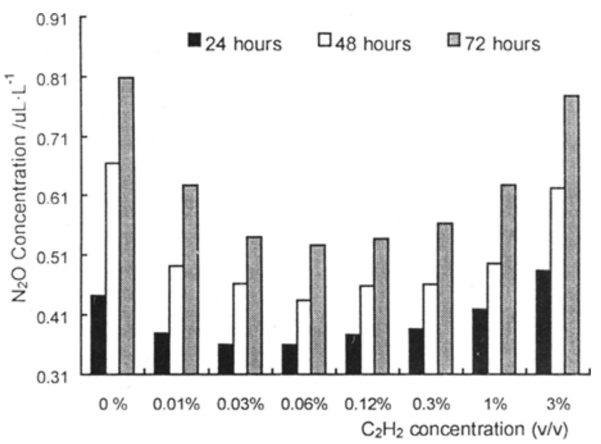


Fig.1. N_2O production under different C_2H_2 concentration with time

48 and 72 h, the best inhibition effect of C_2H_2 appeared at 0.06% at each incubation period. Lower concentration of C_2H_2 than 0.06%, the nitrification was not inhibited completely, while the C_2H_2 concentration was higher than 0.06%, the step of N_2O to N_2 in denitrification was inhibited and N_2O accumulated. Therefore, 0.06% C_2H_2 was the suitable C_2H_2 concentration in this forest soil, and was selected in further incubation experiments in this paper.

Time course analysis of incubation

Rates of N_2O accumulation within incubation bottles were generally linear during the first 72 hours of incubation (Fig.1). In the further experiments, 48 hours was selected as incubation time, at this sampling time, the N_2O concentration in air of headspace was high enough to be detected by GC and accumulation rate of N_2O was still linear.

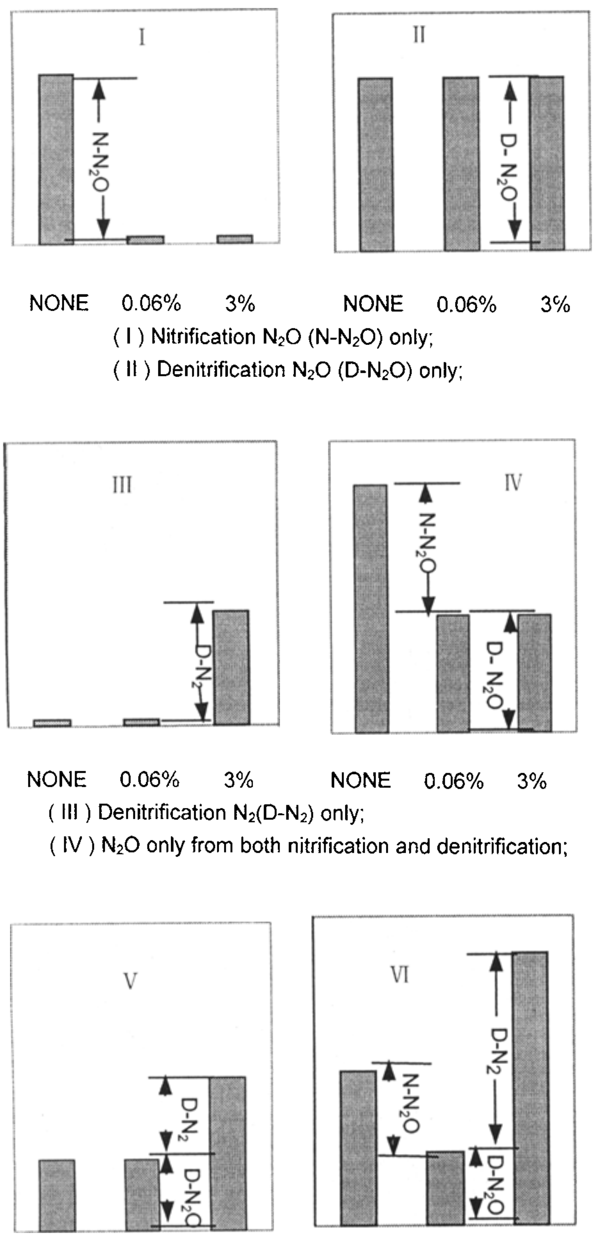
Confirmation of inhibition by change of available nitrogen

At the end of incubation (72 hours), soil NH_4^+-N concentration was 99.2 μL· L^{-1} and NO_3^--N concentration was 149.7 μL· L^{-1} in control treatment (no C_2H_2 treatment). While, NH_4^+-N concentration was 137.8 μL· L^{-1} and NO_3^--N concentration was 26.5 μL· L^{-1} in 0.06% C_2H_2 treatment. Compared with the control treatment, the NO_3^- production and NH_4^+ consumption in soil were inhibited by 0.06% C_2H_2. NH_4^+ is substrate and NO_3^- is product of nitrification.

Combinations of N_2O production under different C_2H_2 concentration treatments

According to different hypothetical responds of N_2O production by Nitrification and denitrification to no C_2H_2, 0.06% C_2H_2 and 3% C_2H_2 treatments, there are six possible combinations (I to VI in Fig. 2) of incuba-

tion results.



(I) Nitrification N_2O (N-N_2O) only;
(II) Denitrification N_2O (D-N_2O) only;
(III) Denitrification N_2(D-N_2) only;
(IV) N_2O only from both nitrification and denitrification;
(V) Denitrification only with both N_2O and N_2 produced;
(VI) All three sources of gaseous N

Fig. 2. Theoretical possible results of C_2H_2 treatment on N_2O production

Production of N_2O under the 0.06% C_2H_2 treatment was assumed to estimate N_2O production by denitrification (Denitrification N_2O), since all autotrophic nitrification should be inhibited by the presence of C_2H_2. The difference between N_2O production under no C_2H_2 and that under 0.06% C_2H_2 was assumed to estimate N_2O production by nitrification (Nitrification N_2O), since any decrease in N_2O production when 0.06% C_2H_2 was added should have been due to inhibition of nitrification. N_2 production by denitrification (Denitrification N_2) was estimated by the difference between N_2O production under 0.06% C_2H_2 and that under 3% C_2H_2.

In our experiments, four types of forest soil at various sampling date from Changbai Mountain were treated with no C_2H_2, 0.06% C_2H_2 and 3% C_2H_2, the combinations of incubation results appeared as II, IV, V and VI (see Fig. 2). These combinations indicated that the denitrification N_2O only (II), N_2O only from both nitrificatison and denitrification (IV), denitrification only with both N_2O and N_2 produced (V), and all three sources of gaseous N (VI). Combinations of I and III were not found in the experiments.

Nitrification and denitrification of forest soils in Changbai Mountain

Both nitrification and denitrification were detectable in mountain dark brown forest soil (Site A) (Fig. 3). Rates of nitrification and denitrification were much higher in July and August than in June and September. The sequence of amounts of different gaseous nitrogen emitted from this soil was denitrification N_2 > nitrification N_2O > denitrification N_2O.

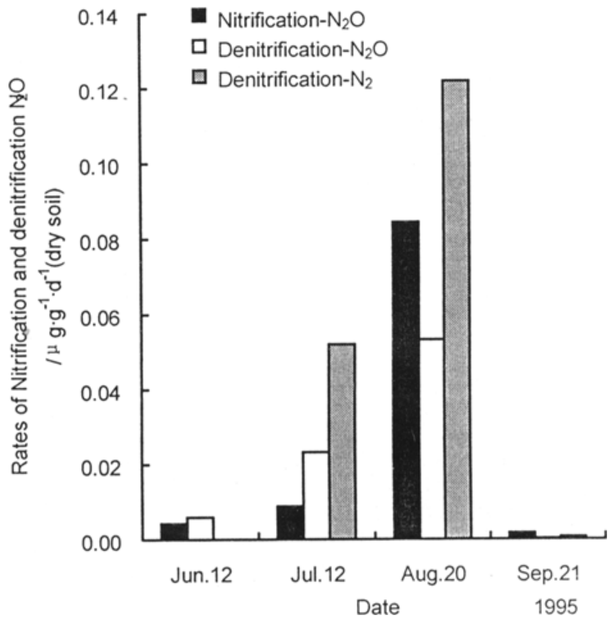


Fig. 3. Distinguishing between Nitrification and Denitrification of Dark Brown Forest Soil (Site A) in Changbai Mountain

Mountain brown coniferous forest soil (Site B) showed the highest nitrification N_2O rate in July, due to the decrease of soil water content (Fig. 4). The soil water content reached its lowest value in July (Xu Hui *et al.* 1999). A dry soil is favorable for nitrification. Comparing with Mountain Soddy forest soil (Site C)

and Mountain tundra soil (Site D), the rate of nitrification of Mountain brown coniferous forest soil in August and September was still high. However, the nitrification N_2O rate in Site C was high only in June and July. The nitrification N_2O of mountain tundra soil (Site D) was almost undetectable. It was the lowest among four soils (Fig. 4).

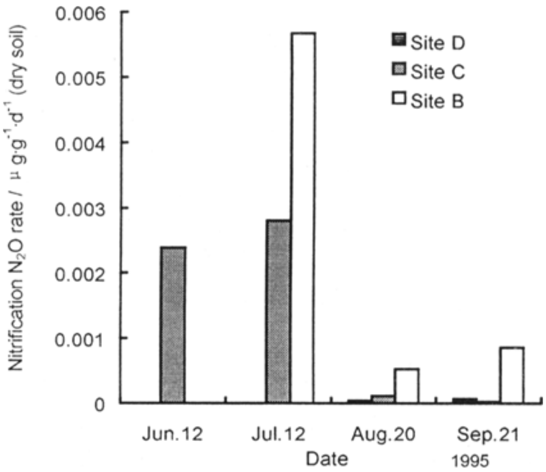


Fig. 4. Nitrification N_2O from different soils in Changbai Mountain

Fig. 5 showed the N_2O production rate by denitrification of soils from three forests (Site B, C and D). Denitrification N_2O rates in these soils were higher in June, July and August. But they were low in September because of low air temperature. The controlling factors for N_2O fluxes from these forest soils in the field experiments are air and soil temperature (Xu Hui *et al.* 1999). There is no significant difference among rates of denitrification N_2O from Site B, C, and D.

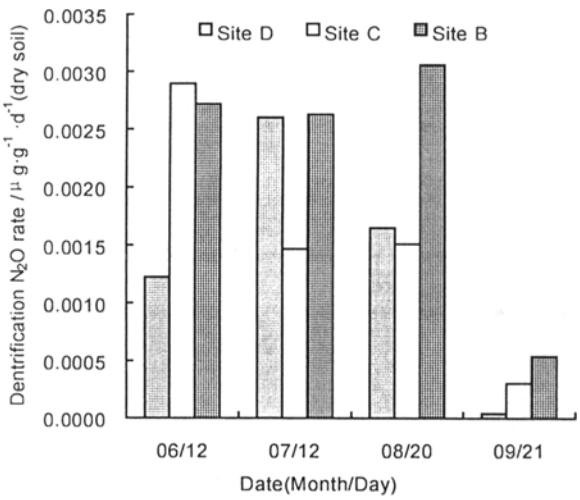


Fig. 5. Denitrification N_2O from different soils in Changbai Mountain

N_2 production by Denitrification in Site B, C and D was shown in Fig. 6. Comparing with nitrification N_2O and denitrification N_2O, the denitrification N_2 rate was much low in these sites. The lowest rates of denitrification N_2 occurred in August in these three sites, when the air temperature was the lowest among those sampling dates. Contribution of denitrification N_2 to gaseous nitrogen emission in these three soils (Site B, C, D) was lower than that in Site A.

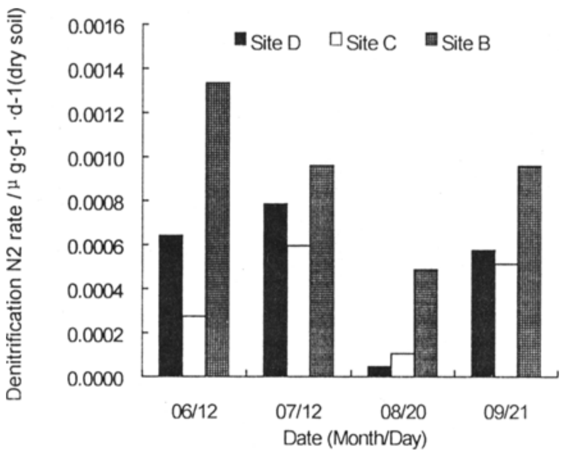


Fig. 6. Denitrification N_2 from different soils in Changbai Mountain

All of three processes, nitrification N_2O, denitrification N_2O and denitrification N_2, were existed in mountain dark brown forest soil (site A), Mountain brown forest soil (site B) and mountain soddy forest soil (site C). Only denitrification (N_2O and N_2) was found in mountain tundra soil (site D). The annually averaged rates of nitrification and denitrification in site A were much higher than that in other three sites. Rates of three soil microbial processes in four forest soils were listed in Table 2.

Table 2. Averaged gaseous nitrogen emissions (N_2O and N_2) from different soils in Changbai Mountain.

Site No.	Gaseous nitrogen emissions /$\mu g \cdot g^{-1} \cdot d^{-1}$(dry soil)		
	Nitrification N_2O	Denitrification N_2O	Denitrification N_2
Site A	248.2×10^{-4}	205.8×10^{-4}	436.5×10^{-4}
Site B	17.6×10^{-4}	22.4×10^{-4}	9.3×10^{-4}
Site C	13.3×10^{-4}	15.4×10^{-4}	3.7×10^{-4}
Site D	0.16×10^{-4}	13.8×10^{-4}	5.1×10^{-4}

The reason for high rates of nitrification and denitrification in site A should be the characteristics of this soil due to the lowest altitude among these four soils. In mountain dark brown forest soil from site A, contributions of different processes to gaseous nitrogen emissions were Denitrification N_2 > Nitrification N_2O > Denitrification N_2. In mountain brown coniferous for-

est soisl from site B, contributions of different processes were Denitrification N_2O > Nitrification N_2O > Denitrification N_2. In mountain soddy forest soil from site C, the same sequence as that of in site B. However, the sequence in tundra soil from site D was: Denitrification N_2O > Denitrification N_2.

Discussion

Two microbial processes contribute most of the emission of N_2O from soil: denitrification and nitrification. The production of gaseous nitrogen by microbial reduction of nitrogsenous oxides (NO_3^-) is known as biological denitrification. Microbial oxidation of NH_4^+ to NO_2^- or NO_3^- is known as nitrification. The nitrification takes place in two steps. In the first one, NH_4^+ is oxidized to NO_2^- with NH_2OH as an intermediate. In the second step, NO_2^- is oxidized further to NO_3^-.

The pathway of denitrification is thought to be:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

The reaction of nitrification can be formulated as:

$$\begin{array}{c} NH_4^+ \rightarrow NH_2OH \rightarrow (NOH) \rightarrow NO \rightarrow NO_2^- \rightarrow NO_3^- \\ \downarrow \\ N_2O \end{array}$$

Until about 1980, denitrification was regarded as the supreme source of atmospheric N_2O, but work reported by Bremner & Blackmer (1981) showed that nitrification could also be a significant source of N_2O. Denitrification is an anaerobic process, while nitrification is aerobic. Their reaction rates usually respond differently to changes in soil conditions and the relative importance of these processes varied with local circumstances. Therefore, many scientists investigate methods that can distinguish these two processes.

Denitrification is difficult to measure directly in the field due to the large concentration of the major product, N_2, in air. Various methods of indirect measurements are available. The most common one is using acetylene as an enzyme inhibitor (the acetylene inhibition method, AIM). Acetylene inhibits the enzymatic reduction of N_2O to N_2. 1 to 10 vol-% is usually sufficient for this to occur (Luo *et al.* 1996). Denitrification can thus be measured as the amount of N_2O produced in soil treated with acetylene. The ratio of N losses as N_2O and as N_2 can be estimated by measuring N_2O emissions from both untreated and treated soils. Use of isotope methods (marking the fertilizer N with ^{15}N and measuring the isotopic enrichment in the products) permits direct measurement of denitrification rates, but it is not in so common use as the AIM method due to cost and difficulty of uniform distribution of added ^{15}N.

Nitrification can be studied by following changes in concentration of NH_4^+, NO_2^- and NO_3^-. Interpretation of such measurements may be difficult, as NH_4^+ and NO_3^- are also produced and consumed by other soil processes. ^{15}N methods can also be used for studies of nitrification, but similar limitations as that for denitrification studies. Another method has been developed in the Nitrification measurement. The first step in autotrophic nitrification is mediated by ammonium oxidase, an enzyme inhibited by acetylene at low pressure (0.001 to 0.01 vol-%). Thus small amounts of acetylene can be used to prevent N_2O production by the ammonium oxiders. This is so called PPM method (use of ppm levels of acetylene to inhibit nitrification). The difference in inhibitory acetylene concentration for ammonium oxidase and nitrous oxide reductase permits estimation of the rates of nitrification, denitrification and N_2O production from each of these processes.

0.01% of C_2H_2 was used firstly by Davidson *et al.* (1986) in measurement of nitrification in forest soil samples. De Boer *et al.* (1992) also found 0.06% was the suitable C_2H_2 concentration in incubation experiments of Dutch forest soil. Since the best inhibition efficiency of 0.06% C_2H_2 on N_2O production in our experiments, 0.06% C_2H_2 was selected to inhibit nitrification in the rest incubation experiments of this paper.

Inhibition results of different C_2H_2 concentration (from 0 to 3%) on N_2O production (Fig. 2) confirmed that the AIM and PPM could be used in nitrification and denitrification measurements of forest soils in Changbai Mountain. Changes in soil available nitrogen concentration during incubation confirmed also the inhibition effect. Additional evidence was also provided by various combinations of N_2O production from different soils under 0, 0.06% and 3% C_2H_2 (Fig. 3). Different responses of soils to the same treatment of C_2H_2 inhibition showed the usefulness of AIM and PPM methods on denitrification and nitrification in these soils.

Gaseous nitrogen (N_2O and N_2) production by nitrification and denitrification was much high in mountain dark brown forest soil than mountain brown coniferous forest soil, mountain soddy soil and mountain tundra soil. The possible reasons for this phenomenon were higher fertility and higher annually mean temperature of mountain dark brown forest soil than other three soils. No nitrification existed in mountain tundra soil is an interesting phenomenon. The soil type, vegetation and mean annual temperature of mountain tundra soil is much different from other soils. However, which factors controlling ratio of nitrification and denitrification among these soils are unclear, and more studies are needed.

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